CHARACTERIZATION OF A WHEAT MAPPING POPULATION FOR GROWTH PATTERN AND STUDYING STAYGREEN WHEAT CANOPY USING MULTISPECTRAL UAV IMAGES

A Thesis

by

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ABSTRACT

Uniform tiller distribution and plant architecture are crucial traits that are directly linked to the length of vegetative and reproductive phases. Minimum vegetative growth and an earlier synchronized flowering pattern result in a prolonged grain filling period. In determinate plants, minimum vegetative growth leads to sustained photosynthesis during the grain filling phase and produces sufficient assimilates to maximize size and weight of grains. Indeterminate growth has undesirable attributes including a sustained sequence of tillers and non-uniform flowering which lead to variation in maturity. Therefore, an indeterminate tillering pattern is not advantageous for harvesting. In this study, UAV multispectral imagery was used to monitor spectral reflectance patterns of plants at post flowering stages. Determinate plants with staygreen phenotypes showed a low rate of senescence and produced high grain yield in contrast to indeterminate lines. A positive correlation was seen between grain yield and vegetation indices such as NDVI, GNDVI and NIR/Green ratio at all reproductive stages.

DEDICATION

This thesis is dedicated to my teachers, my parents, my aunt, my siblings, my cousins, my friends and all those people who are my inspiration. Thank you all for your guidance, moral support and encouragement.

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CHAPTER I

INTRODUCTION AND OBJECTIVES

1.1. Background of study

In light of the ever-growing world population**,** agricultural crop production will need to be doubled by 2050 (Araus et al., 2003). Climate change and extreme weather conditions are potential threats to food supply and food security (FAO 2011). In terms of severe climatic changes, heat and drought stress have had large impacts on agricultural production in most areas of the world (Kang et al., 2008). According to The Intergovernmental Panel on Climate Change report (IPCC 2014), an increase in unfavorable conditions may result in significant economic losses in agricultural regions. Therefore, to cope with these challenges, climate adaptation and mitigation measures for crop production systems are needed by plant breeders and crop scientists.

Under environmental stress, biochemical signals for early flowering cause prevention of further increase in branching by suppressing vegetative meristem as an adaptive response. This mechanism results in uniform tillering and early flowering. The determinate growth habit promotes grain filling, a time during which sustained photosynthesis leads to an increase in grain size and weight. In contrast, profuse tillering leads to variations in flowering and maturation timing, as well as grain and panicle size. Therefore, this is also an undesirable attribute for harvesting (Azam et al., 2002). In response to environmental conditions (light, temperature and water etc.) plants develop a canopy structure and intercept light accordingly, converting solar radiation into photosynthate and distributing assimilates between different plant components **(**Keating, 1993**).** Shoot architecture affects the light harvesting potential, flowering synchronicity and seed setting that determine a plant's reproductive success. Canopy structure affects proportion of sunlight intercepted, and canopy temperature also varies with the view angle (Jones et al., 2009). There is a potential to improve radiation interception by promoting more erect leaf angles, fast early leaf area growth and by extending the duration of green leaf area as the crop matures over the crop cycle. Extended photosynthetic leaf area duration is considered to be a beneficial trait termed functional staygreen (Martin et al., 2011). The "staygreen" trait results in the availability of more photosynthetic tissue for further assimilation and more water extraction from the soil. Under heat stress conditions, reduced photo-assimilation during grain filling can limit grain yield (Spano et al., 2003). Therefore, traits related to radiation-use efficiency, such as photosynthetic rate, and staygreen can play an important role in stress conditions. Loss of chlorophyll and a change in a:b ratio due to premature leaf senescence was found to be associated with a decline in the rate of photosynthesis, which reduced the wheat grain yield during the grain filling stage. Genetic variation for the timing and rate of leaf senescence was found between species and genotypes (Reynolds et al., 2011).

High yielding and environmentally adapted plants can be selected more efficiently by connecting genotype to phenotype. However, lack of access to phenotyping capabilities limits our ability to dissect the genetics of quantitative traits related to growth, yield and adaptation to stress. Use of high throughput phenotyping techniques can better eliminate bias between data and experimental repeats than conventional techniques that are based on visual scoring by experts with less accuracy (Prasad et al., 2008). High throughput phenotyping techniques may reduce the limitations in exploring genetics of quantitative traits related to plant growth, development, stress responses, physiology and architecture (Rosnell and Honkavaara, 2012).

Numerous studies have employed multispectral data to monitor crop growth, yield and biomass estimation using vegetation spectral reflectance parameters (Lopresti et al., 2015). The solar radiation that a leaf absorbs is directly related to photosynthetic activity. The red, green and blue light (in the region of 400 to 700 nm) is absorbed by the plant to convert water and carbon dioxide into nutrients while infrared light is the leftover. Near infrared spectroscopy has been used to study crop growth and yield performance under stress conditions (Rosnell and Honkavaara, 2012). Spectral reflectance information from leaves or canopies is used to generate vegetation indices. Vegetation indices are algebraic combinations of different spectral bands that provide information about vegetation vigor and different plant characteristics, such as a plant's photosynthetic active biomass, water status and chlorophyll content (Hatfield et al., 2008). The normalized difference vegetation index (NDVI) and green normalized difference vegetation index (GNDVI) are two of the of the most commonly used vegetation indices as they, provide information related to nitrogen status, chlorophyll content, green leaf biomass and grain yield only on the basis of NIR and red or green band ratio (Schepers et al., 2003).

1.2 Rationale and objectives

The *objective* of this study is to explore morphological and physiological features of plant growth with respect to a plant's canopy distribution, vegetation reflectance and estimation of grain yield and quality attributes using UAV based multispectral images. The ***central hypothesis*** of this proposed study is that uniform tiller distribution is a crucial trait that is directly linked with the length of vegetative and reproductive phases of a plant. These developmental and growth stages are dependent on the varying thermal requirements of a plant and these requirements vary according to each individual stage of plant.

UAV analysis could be helpful in identifying the relationship of the wheat plant's morphology with thermal requirements of individual genotypes at different growth and developmental stages. The current study deals with the processing of multispectral UAV imagery to derive vegetation indices to study the relationship between plant growth pattern, grain yield and end use quality. The advantage of utilizing UAVs in agriculture is being able to see a field in its entirety, which can be time consuming and unrealistic for farmers to do on foot. The fields can be viewed as frequently as desired and at a lower cost than utilizing a manned airborne platform or satellite imagery. Combining traditional crop simulation features with modern technologies and strategies of research will be helpful in exploring the potential factors affecting the growth and development of the wheat plant. By optimizing these potential factors for yield and quality in response to a changing climate or environment, we expect the best crop yield in future conditions. The specific objectives of this dissertation were to 1) Identify the link between determinate reproductive inflorescence and yield quality stability and 2) Determine the relationship between the staygreen and reproductive determinacy with respect to grain yield using remote sensing.

CHAPTER II

SPECTRAL REFLECTANCE-BASED PHENOTYPING TO STUDY STAYGREEN WHEAT CANOPY IN RELATION TO REPRODUCTIVE DETERMINACY AND AGRONOMIC TRAITS

2.1 Introduction

Heat stress tolerance is associated with photosynthesis maintenance, chlorophyll retention, availability of carbohydrates during sink development and prolonged grain filling duration (Hays et al., 2007). According to the timing and duration of vegetative and reproductive growth, plants can be determinate or indeterminate. Plants that complete their vegetative growth prior to the start of flowering have been termed “determinate plants.” Indeterminate plants display simultaneous vegetative growth and reproductive growth, producing vegetative parts along with flowers and filling grain on other branches (Russell 1985). Indeterminate crops have excessive vegetative growth accompanied by varying flowering and maturation timing of spikes within the same plant. Indeterminacy is unfavorable in terms of final grain yield and harvesting as later produced tillers may or may not be productive (Squire and Azam, 2002). In the case of determinate growth behavior, flowering is synchronized within the plant and the grain filling period is longer, as the plant stops its vegetative growth before the start of flowering. Long grain filling periods have been associated with higher yields in determinate crops (Stützel and Aufhammer, 1992). In determinate crops, the assimilate supply and prolonged grain filling can be ensured by delaying leaf senescence and continuing photosynthesis at the later point in the crop season (Thomas and Howarth, 2000; Spano, 2003).

Delayed leaf senescence is the main feature of the staygreen phenotype, which has greater green leaf area than senescent lines and which is advantageous in terms of maintaining photosynthesis and assimilate supply to the developing grain (Christopher et al., 2016). The staygreen phenotype can be either functional or nonfunctional depending upon photosynthetic competence. In the case of functional staygreen, retention of chlorophyll is associated with continuous photosynthesis, improved grain filling efficiency, high carbohydrate reserves in stem and high grain weight. In contrast to this, the non-functional staygreen type has persistent green leaf color due to lesions in the chlorophyll recycling process. Consequently, there is no advantage in terms of yield as it lacks photosynthesis competence (Borrell et al., 2000; Thomas and Howarth, 2000). Thus, in the context of abiotic stress adaptability, only the functional staygreen phenotype has a potential to be used in crop improvement programs because it can sustain photosynthesis through morphological and physiological changes (Christopher et al., 2016). Different methods including visual scoring for whole plant senescence, SPAD meter measurements for number of green leaves per culm, and extent of flag leaf greenness during grain filling have been employed in previous studies to asses the staygreen phenotype in the field (Jordan et al., 2012). However, the morphological and physiological features of the staygreen trait are yet to be fully explored by robust phenotypic techniques (Lopes and Reynolds, 2012). The onset of senescence varies between genotypes, and the date of measurements can impact results (Christopher et al., 2014). In recent studies, the periodic NDVI pattern measured by Greenseeker instrumentation showed a strong association with senescence dynamics. These measurements assessed the rate and duration of greenness loss and have been used to quantify components of the staygreen phenotype in the field (Christopher et al., 2016). staygreen trait-related measurements at late crop cycle with respect to leaf senescence are fitted to thermal time of anthesis of each genotype (Harris et al., 2007). More recently, spectral reflectance-based vegetation indices have been used to identify the expression patterns of staygreen related to plant phenology, while the use of spectral reflectance based indices have also been reported independently of phenological stages of the plant (Lopes and Reynolds, 2012).

Various indices have been developed for the assessment of chlorophyll content in leaves. Green normalized difference vegetation index (GNDVI) is used to precisely determine chlorophyll content (Castro and Sanchez 2008). The NDVI is less sensitive to chlorophyll content when the canopy is moderate or dense, so it is best applied in sparse canopies (Gamon *et al.*, 1995). GNDVI is a modified form of normalized difference vegetation index that is derived using the green band instead of the red band, as in case of NDVI. The GNDVI is more sensitive to chlorophyll content than NDVI when the leaf area index is high. Therefore, GNDVI can overcome the problem of saturation, which NDVI can exhibit at later growth stages (Gitelson and Merzlyak, 1998; Mashaba et al., 2016). Leaf senescence is genetically controlled and also influenced by environmental conditions, which results in chlorophyll breakdown, decreased chlorophyll a/b ratio, reduced photosynthesis and remobilization of nutrients to different parts of the plant (Gitelson and Merzlyak 1998). Some of the consequences of leaf senescence onset can be visually detected. When chlorophyll degrades, reflectance in the region of the visible spectrum (400-700 nm) increases due to an increased number of scattering surfaces, while the later phase of senescence results in low NIR reflectance due to mesophyll destruction due to destruction of mesophyll layer (Castro and Sanchez 2008).

In this study we have used multispectral unmanned aerial vehicle (UAV) acquired images to derive vegetation indices (VIs), such as NDVI and GNDVI, which enabled us to study plant health, canopy cover, chlorophyll content and abiotic and biotic stress responses. The NDVI values ranges from -1 to +1, indicating non-vegetative features (water, barren areas, ice or snow etc.) and increasing greenness, respectively (Geipel et al., 2016). The aim of multispectral UAV image use is to monitor plant growth and measure a staygreen phenotype quantitatively through the interaction between light and plants while maintaining better temporal and ground resolution.

2.2 Material and methods

2.2.1 Plant material and study site

The study was conducted in a College Station, TX. green house trial and on a wheat field in Corpus Christi, TX. The field was divided into 364 individual plots 5×10 ft. in size. A Halberd and Len RIL population (comprising 180 RILS and two parent lines), replicated twice in an alpha lattice design, were grown in the field. Climatic conditions at the study site were mild to progressively increasing throughout the growing period (Table 2.1). Maximum average temperature during flowering to grain filling stage was 33-34 ℃ with maximum average rainfall 6.54 inches and 5.99 inches at late vegetative growth and late grain filling stage respectively.

Table 2.1 Temperature and rainfall pattern during crop growth season.

Temp/month	Jan	Feb	March	April	May
Max temp (℃)	31.11	29.39	33.89	34.39	33.89
Min temp (℃)	0.00	1.11	8.89	10.00	14.39
Average temp (℃)	13.56	17.06	21.42	23.72	26.47
Total precipitation (inch)	2.08	0.21	6.54	3.4	5.99

2.2.2 Field and greenhouse trait measurements

Number of days to heading (50% plants exhibiting heading out), flowering (50% pollinated spikes) and physiological maturity (50% of the spikes in a plot showed loss of greenness) were recorded according to method described by Zadocks et al., 1974. RILS were characterized into determinate and indeterminate types according to the duration of completion of their vegetative and reproductive growth. Staygreen lines were identified among the determinate group based on flowering time, rate and duration of leaf senescence and physiological maturity. The timing of greenness loss at the late reproductive stage was also studied to identify RILs with the staygreen trait in the greenhouse trial. Data for grain yield (per square ft.), grain weight (mg), grain diameter (mm) and grain moisture (%) were collected from staygreen and non-staygreen plants.

2.3 Image acquisition and processing

The study included the monitoring of wheat crop growth at different stages with high resolution multispectral RGB and NIR images acquired from an UAV. The false color images acquired by the sensor are in the region of green, red and NIR that are equivalent to Landsat Thematic Mapper bands TM2 (green), TM3 (red) and TM4 (NIR) respectively.

Table 2.2 (a) Ground resolution and view of field for images gathered at various altitudes above ground (b) Sensor and lens parameters.

(a)

Object distance (Altitude above ground level)	Ground Resolution in mm per pixel
122 m	72.36
213.4 m	126.54
365.8 m	216.91

(b)

Sensor Dimension	6.59 × 4.9 mm
Pixel Size	5 micron
Camera Lens Focal Length	8.43 mm

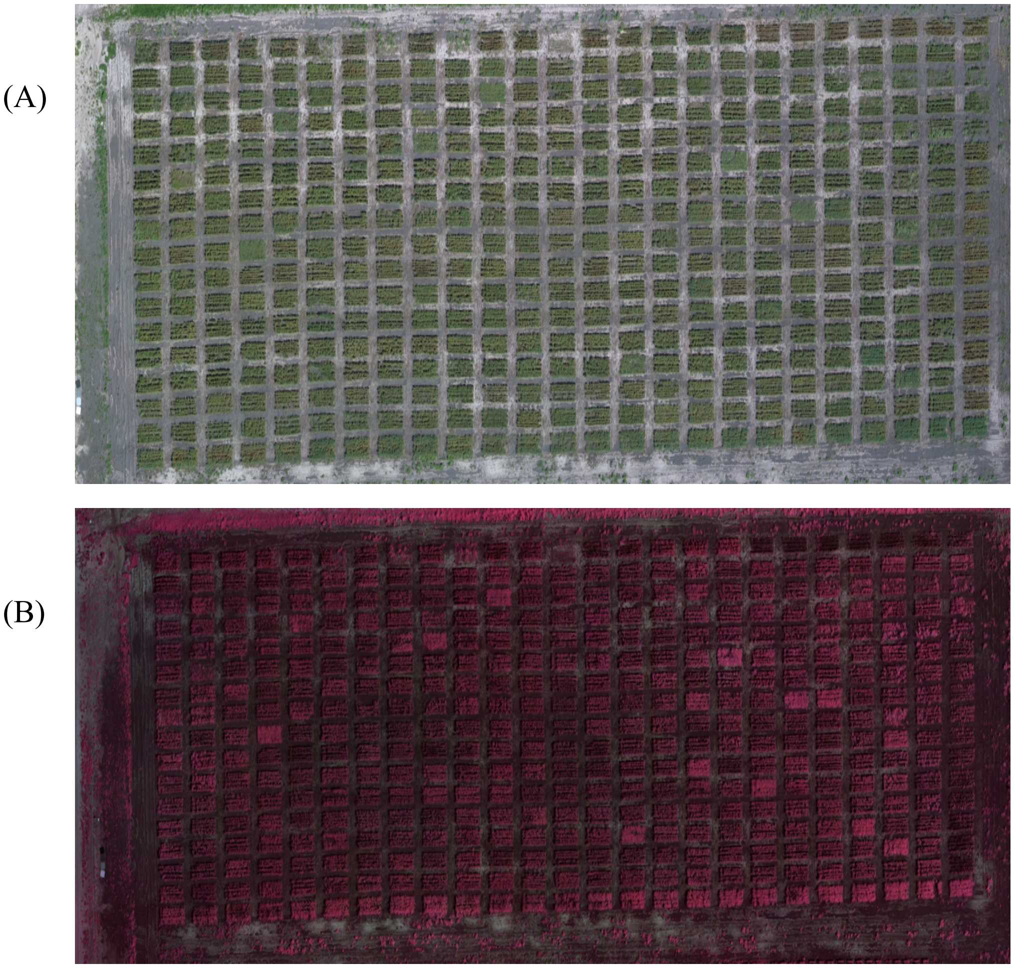

Figure 2.1 (A) RGB UAV image of wheat field, (B) NIR UAV image of field; taken at post flowering stage in current study.

For color images, the response of the sensor to the different bands are in red, green and blue regions of the visible spectrum. Flying height was 30 m above ground. The specifications of the sensor are given in Table 2.2. The ADC Snap sensor has the ability to capture images that are free of motion blur. The field surveys were conducted throughout the wheat crop growth period and RGB and NIR images were collected at each individual stage (Fig. 2.1).

2.3.1 Conversion of digital numbers into reflectance

To derive biophysical vegetation parameters, the images need to be adjusted by radiometric correction, as recorded digital numbers (DNs) possess the values for both the spectral characteristics of vegetation or soil and environmental conditions (Campbell and Wynne, 2012). Thus, the observed digital brightness value might be the result of a portion of surface reflectance as well as the part of the atmospheric scattering. Hence, the basic purpose of radiometric correction is to identify the actual surface reflectance brightness. One of the approaches used to correct remotely sensed images radiometrically, is to use target objects of known brightness spectra in the field (Laliberte et al., 2011). Therefore, measurements from acquired images can be compared with that of target objects whose brightness values are recorded at the time of image acquisition (Campbell and Wynne, 2012). The approach we used in our study is the ground-based *in situ* measurements of reflectance of a calibrated target. Four calibration targets (each 0.6 m × 0.6 m) with reflectivity 3%, 12%, 33% and 56% were placed within the flight path of a UAV platform. A laboratory spectrophotometer, the “Perkin-Elmer Lambda 1050,” was used to calculate reflectance of calibrated targets and selected vegetation and soil samples during the time of image acquisition. The ASD field spectrophotometer was first calibrated using a Spectralon (registered trademark of Labsphere, Inc.) for reflectance standard. The empirical line calibration method (Wehrhan et al., 2016) enabled us to derive coefficients required to fit the DNs of the Tetracam ADC snap multispectral imagery to the actual measurements of ground spectral reflectance. Images were processed in ENVI software to extract reflectance values from the field plots.

2.3.2 Vegetation index calculation

The broadband spectral reflectance data of UAV images were used to determine the vegetation indices (Table 2.3) for each plot representing an individual RIL.

Table 2.3 The vegetation indices calculated in this study.

Index	Equations	References
Red NDVI	NIR-Red/NIR+Red	Jiang et al., (2006)
Green NDVI	NIR-Green/NIR+Green	Gitelson et al. (1996)
Ratio	NIR/Green	Sripada (2006)

2.4 Results

2.4.1 Conversion of digital numbers into reflectance

The empirical line approach produced linear relationships between DNs and ground reflectance for each band using four calibration panels per image (Table 2.4).

Table 2.4 Regression equations and coefficients derived by empirical line calibration method to fit uncalibrated images where x is representing DNs of the UAV image.

		NIR	Red	Green
Image 1	Regression equation	y = 0.4631x - 4.6207	y = 0.469x + 0.128	y = 0.3434x + 0.5801
	R-Squared	R² = 0.99017 NIR	R² = 0.99519 Red	R² = 0.99611 Green
Image 2	Regression equation	y = 0.4397x - 6.8496	y = 0.3785x + 0.272	0.3426x - 1.8763
	R-Squared	R² = 0.99905	R² = 0.99753	R² = 0.99845
Image 3	Regression equation	y = 0.6355x - 7.0226	y = 0.5393x + 3.0104	y = 0.4915x + 1.2339
	R-Squared	R² = 0.99948	R² = 0.99978	R² = 0.99855
Image 4	Regression equation	y = 0.741x - 11.134	y = 0.5426x + 3.5568	y = 0.4844x + 2.3111
	R-Squared	R² = 0.99884	R² = 0.99869	R² = 0.99935
Image 5	Regression equation	y = 0.7683x - 5.4045	y = 0.6459x + 6.0163	y = 0.5566x + 5.1393
	R-Squared	R² = 0.99696	R² = 0.98249	R² = 0.9886
Image 6	Regression equation	y = 0.484x - 5.127	y = 0.3791x + 1.8302	y = 0.3218x + 0.375
	R-Squared	R² = 0.99872	R² = 0.99775	R² = 0.99874

2.4.2 Vegetation indices from spectral reflectance

The spectral reflectance patterns of the wheat crop illustrated changes in visible and near infra-red wavelengths regions during all growth and developmental stages. These changes were correlated to VIs calculated using multi-temporal spectral reflectance information from visible and NIR wavelengths (Figure 2.2. a, b, c).

(A)

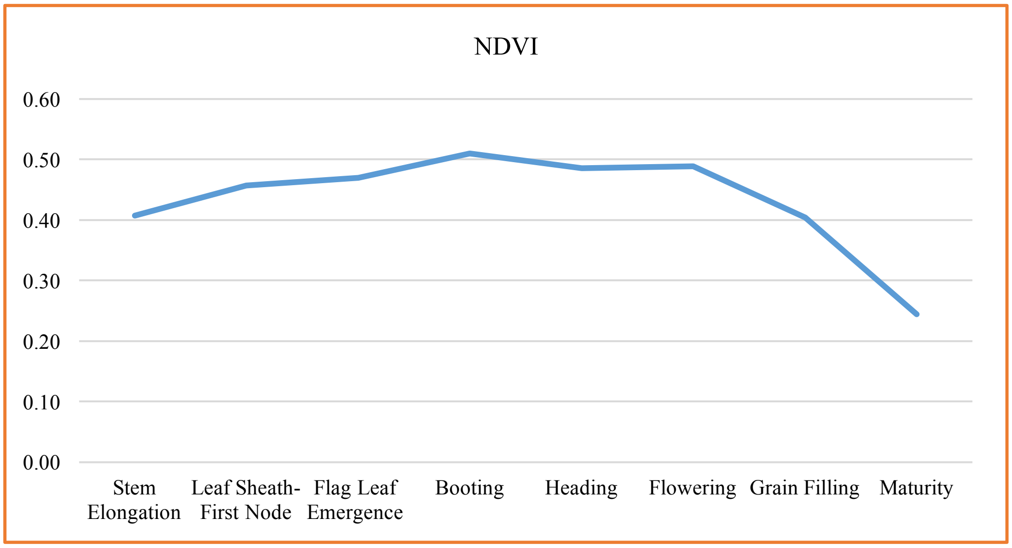

Figure 2.2 Change in vegetation indices in Hal×Len RILS population from early vegetative growth to late reproductive stage. (A) Gradual increase in NDVI from stem elongation with maximum value at booting stage, while at heading and flowering stage there is a fluctuation in trend. There is a decrease in NDVI at late reproductive phase due to onset of senescence. (B) Continuous moving trend was seen in greenness index (GNDVI) throughout the growth period from vegetative to reproductive stages. (C) Similar moving trend was seen in ratio index (NIR/Green) from vegetative to reproductive stages.

Figure 2.2 Continued.

The relative VIs trending lines of RILs revealed that there was a similar response of gradual increase in NDVI values during the early vegetative growth period. This change in NDVI values continued with the increasing trend up to the onset of the reproductive stage and peaked at the booting stage. A slight decrease in NDVI was seen at the heading stage, which again changed with a minor increase at the flowering stage followed by a continuous decline in post-flowering stages. The GNDVI and NIR/Green measurements fluctuated with sudden rises and falls at different growth stages throughout the crop growth cycle.

(A)

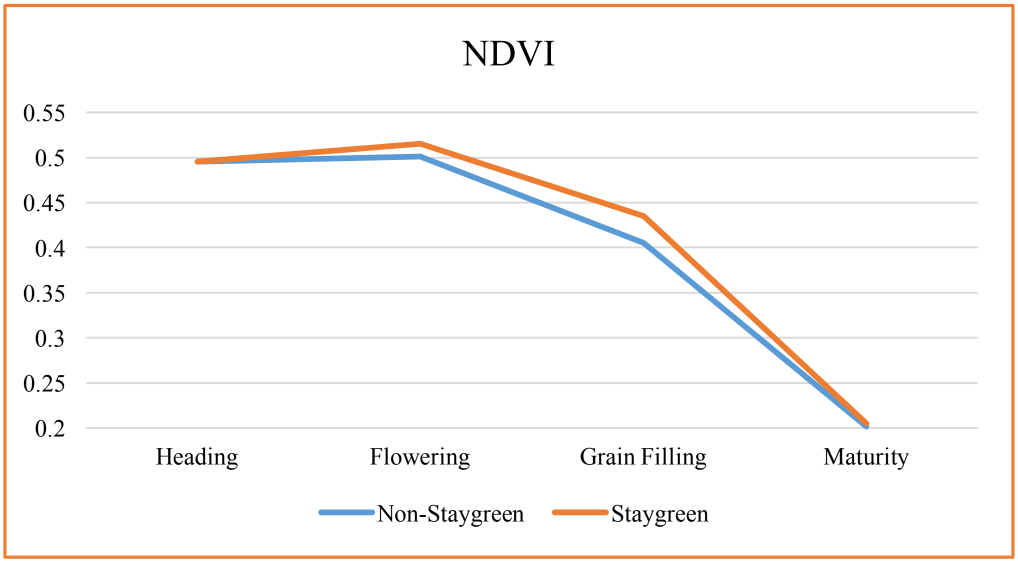

Figure 2.3 Validation of spectral reflectance based measurements with staygreen phenotyping. (A) Rate of decline in NDVI during late reproductive stages in staygreen and non staygreen lines. (B) Rate of greenness index (GNDVI) staygreen and non staygreen lines. (C) Rate of NIR/Green decrease.

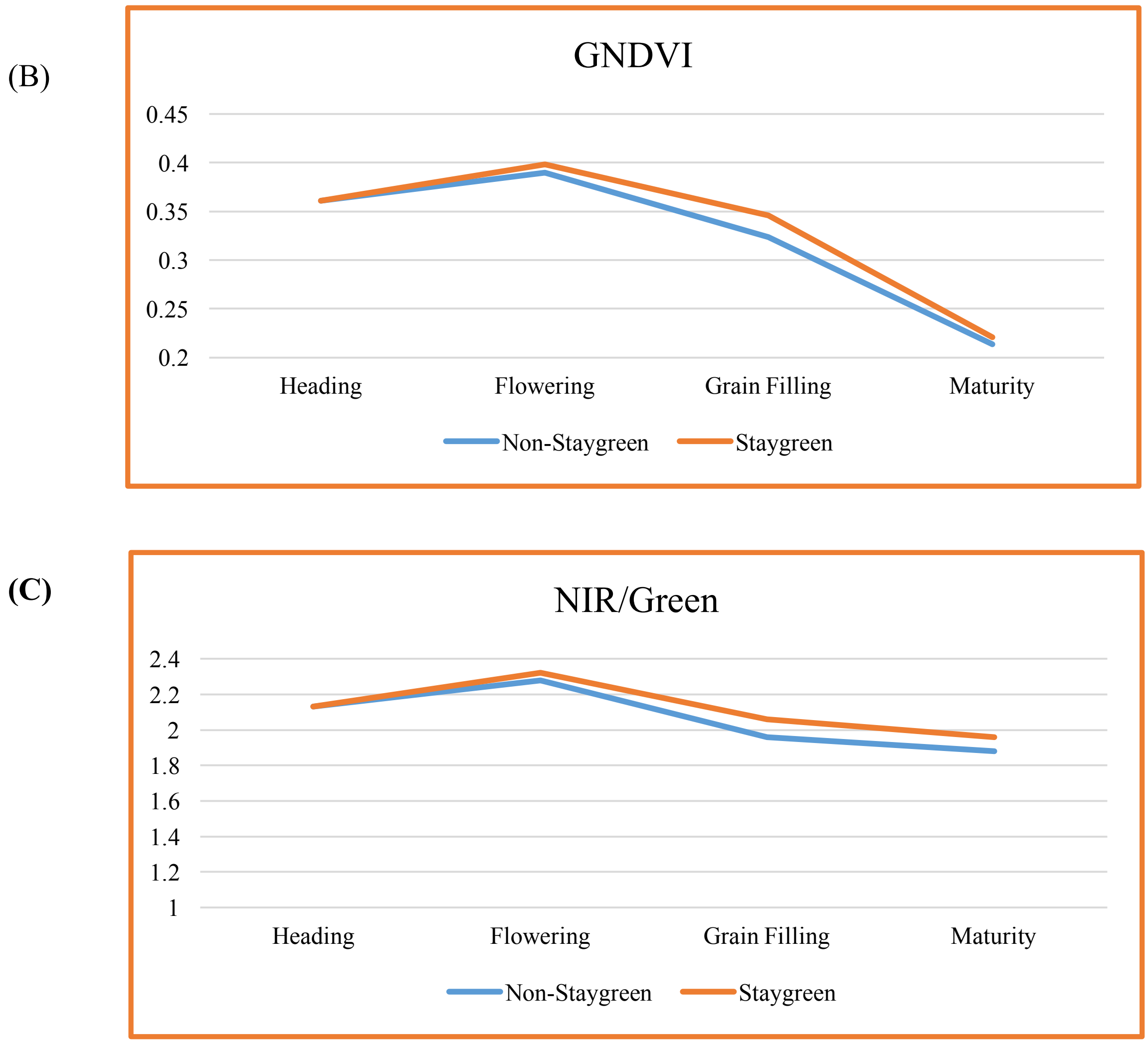

Figure 2.3 Continued.

An association was observed between morphological characteristics of staygreen plants and their spectral reflectance information. At the post-flowering stage, when the VIs values started to decrease after reaching at peak at the booting stage, the rate of decline in NDVI and greenness index (GNDVI) was slow in staygreen types as compared to non-staygreen plants (Fig 2.3). The difference in rate of VIs decline was narrowed as the senescence progressed. The visible and NIR images had shown the maintenance of green leaf area during post flowering and grain filling stages in case of staygreen lines.

2.4.3 Grain yield and quality parameters

The average grain yield and quality parameters data in staygreen and parent lines recorded at time of harvest are presented in table 2.5.

Table 2.5 Grain yield, weight, diameter and grain moisture content of staygreen lines and parents Halberd and Len.

Lines	Grain yield kg/acre	Single Kernel weight (mg)	Grain diameter (mm)	Grain moisture (%)
SG53	515.12	21.68	2.365	13.275
SG63	502.00	20.615	2.315	21.065
SG64	420.00	21.1	2.435	12.165
SG123	133.00	23.1	2.405	12.465
SG124	512.00	21.84	2.38	12.74
SG152	505.00	20.825	2.305	12.89
Halberd	276.00	21.82	2.398	12.42
Len	238.45	20.495	2.332	12.135

2.4.4 Association between traits

In SG lines, grain yield was highly associated with NDVI, GNDVI and NIR/Green at late vegetative to all reproductive growth stages. An especially high correlation was noticed at booting stage ($P \leq 0.05$) (Table 2.6). Interestingly, the staygreen types were highly tolerant for severe effects caused by periodic rainfalls at later stages of maturity and before harvesting.

Table 2.6 Pearson's correlation coefficients of vegetation indices and single kernel parameters

* = significant, NS= not-significant.

	Grain Weight	Grain Diameter	Grain Moisture	Grain Yield
(a) Booting				
NDVI	0.19^{NS}	0.44^{NS}	-0.23^{NS}	0.71*
GNDVI	0.22^{NS}	0.36^{NS}	-0.13^{NS}	0.68*
NIR/Green	0.22^{NS}	0.35^{NS}	-0.13^{NS}	0.67*
(b) Heading				
NDVI	0.25^{NS}	-0.08^{NS}	0.37^{NS}	0.54^{*}
GNDVI	0.17^{NS}	0.14^{NS}	0.22^{NS}	0.45^{*}
NIR/Green	0.17^{NS}	0.14^{NS}	0.21^{NS}	0.45^{*}

Table 2.6 Continued.

	Grain Weight	Grain Diameter	Grain Moisture	Grain Yield
(a) Flowering				
NDVI	0.10 NS	0.13 NS	0.26 NS	0.57*
GNDVI	-0.04 NS	0.31 NS	0.11 NS	0.54*
NIR/Green	-0.05 NS	0.32 NS	0.10 NS	0.54*
(b) Grain Filling				
NDVI	0.60 NS	-0.04 NS	0.12 NS	0.55*
GNDVI	0.43*	0.08 NS	0.16 NS	0.61*
NIR/Green	0.43*	0.08 NS	0.15 NS	0.61*

2.5 Discussion

The change in wheat canopy reflectance spectra can be observed for different growth and developmental stages (Fig. 2.2). As plants grew, the curve for NDVI values increased initially due to increased vegetation ground cover. At the booting stage, the NDVI values reached the highest point of the season. However, at later stages VIs values started to decrease due to decreased NIR reflectance and increased reflectance in visible regions. At late stages, less absorption by pigments caused the increased reflectance in the visible spectral region while NIR reflectance decreased due to senescence. This reverse mechanism of absorption and reflectance in later stages resulted in decreased indices values, which were associated with the onset of leaf senescence (Castro and Sanchez-Azofeifa, 2008). Different studies have employed spectral reflectance data of different growth stages to estimate and predict the final yield on the basis of canopy vigor and health (Chang et al., 2005; Jin et al., 2013). However, in the case of non-staygreen lines, the multiple regression and correlation analysis using vegetation indices at different growth stages did not show significant positive correlation between VIs and final grain yield in our study except for the grain filling stage, where yield was associated with greenness index (GNDVI and NIR/Green). The possible reason for not being able to validate spectral reflectance data with observed grain yield in non-staygreen may be due to the contribution of environmental conditions at the maturity stage, which affected the actual grain yield before harvesting. Despite the unfavorable weather conditions at the late reproductive stage, the staygreen lines were highly tolerant of these environmental effects; In particular, lodging resistance was seen under heavy rainfall conditions. Therefore, the harvested grain yield showed high correlations with NDVI and greenness indices (GNDVI and NIR/Green) at different growth stages. At the booting stage, positive correlation was found between vegetation indices and grain yield (NDVI=0.71, GNDVI=0.68 and NIR/Green=0.67) ($P \leq 0.05$).

Delayed senescence is the main feature of the staygreen genotype, which results in maintaining the chlorophyll for a longer period of time as compared to non-staygreen genotypes. Staygreen lines maintain the green color of the flag leaf while the stem becomes senescent. This stands in contrast to non-staygreen types, where senescence occurs in flag leaves along with the stem in the case of the normal senescence process (Borrell, et al., 2104). Staygreen plants have different types with respect to time of occurrence and rate of senescence (Spano et al., 2003). The senescence phenomenon is defined by the genetics of a plant, but it is also influenced by environmental conditions, which cause the destruction of chlorophyll. The photosynthetic chlorophylls and carotenoids absorb light in the visible region of spectrum (400-700nm). Plants have two types of chlorophyll, chlorophyll *a* and chlorophyll *b*, while beta-carotene is main class of the carotenoid group. These pigments are responsible for energy absorption in plants. Chlorophyll *a* absorbs light in the 430-450 nm and 640 nm-680 nm portions of the electromagnetic spectrum while chlorophyll *b* absorbs energy in 430-480 nm and 630-660 nm wavelength regions (Araus et al., 2001).

Chlorophyll is responsible for greenness of leaves as it absorbs red and blue light while green light is reflected back; Therefore, plants appear green. Carotenoids absorb light in the blue-green and violet regions and reflect light in the yellow, red and orange regions of the spectrum. When a plant reaches maturity, a reduction in chlorophyll is the indication of initiation of leaf senescence. Plants engineered for overproduction of chlorophyll by overexpressing the gene encoding the chlorophyllide *a* oxigenase have shown a delayed senescence (Kusaba et al., 2007). Healthy plants have a higher concentration of chlorophyll *a* and *b* while stressed or senescent vegetation appears more yellow because of increased amount of carotenoid pigments (Carter and Knapp, 2001).

NDVI and GNDVI and other indices that are based on NIR and green wavelengths have been used to describe the relative content of chlorophyll during the grain filling to maturity stage. Delayed leaf senescence and a low rate of chlorophyll reduction in staygreen plants maintains the supply of carbon assimilate during the grain filling stage, which results in maximizing the mass per grain. Our results showed that there is a positive correlation between vegetation indices and grain weight at the grain filling stage (Table 2.6). Moreover, the staygreen genotypes in wheat and sorghum have shown improved yields under abiotic stress conditions at the post-flowering stage as compared to genotypes lacking this trait. In a recent study, the staygreen trait was highly associated with grain yield and quality depending on certain environmental conditions (Lopes and Reynolds, 2012). In our results (Table 2.6), the average grain yield and relative grain quality parameters (diameter and weight) values are higher in SG lines as compared to parent lines, for the exception being RIL 123, which had comparatively lower grain yield among staygreen lines. This is possibly due to the presence of non-functional staygreen characteristics.

The functional staygreen plant has the ability to preserve chlorophyll and continue to photosynthesize until late stages of development. The rate of senescence was lower (Lopes and Reynolds, 2012) than non-staygreen types, resulting in higher yield. In contrast, non-functional staygreen phenotypes conserve their chlorophyll only due to defects in chlorophyll degradation pathways, and they lack photosynthetic competence. Therefore, this staygreen does not contribute to yield. The observations in this and previous studies suggest that determinate and functional staygreen plants maintain photosynthesis for a longer period of time and exhibit a longer grain filling period, and have the potential to produce improved yield as compared to non staygreen plants.

2.6 Conclusion

The staygreen determinate lines have consistently more duration and photosynthetic activity during the grain filling period as compared to parent lines and non-staygreen determinate lines. The staygreen trait is coupled with maintenance of photosynthesis at late stages, which is associated with high grain yield. Although late rainfall caused lodging of the crop, staygreen lines were resistant to this stress. This spectral reflectance-based study with high temporal resolution provides validation of staygreen behavior at the post-flowering stage. However, broadband wavelength based indexes alone provide information only about greenness of leaves, while photosynthetic activity could not be estimated unless it was correlated with actual grain yield. To study functional and non-functional staygreen plants differentially for biochemical and biophysical properties, narrow band vegetation indices will be needed to provide clearer estimations in association with other direct physiological measurements.

CHAPTER III

IDENTIFYING THE LINK BETWEEN DETERMINATE REPRODUCTIVE INFLORESCENCE AND YIELD STABILITY

3.1 Introduction

Increasing food security challenges demand for development of a crop production system that can better cope with the severity and climatic extremes present in many parts of the world today (Trnka et al., 2014). In the past, various crop models have been presented which describe ideotype as a model plant associated with greater quantity and quality of grains (Donald 1968). “Ideotype” is defined as the combined effects of morphology, physiology and genetic makeup of a plant that determines the optimum crop performance in a given environment (Martre at el., 2015). Bread wheat *Triticum aestivum* (2n= 6X =42, AABBDD) has various potential genetic resources for grain yield and quality improvement due to the constitution and number of chromosomes it has. It displays numerous forms of morphological traits in different environments and natural selection processes (Wang et al., 2016).

The wheat ideotype was first time proposed by Donald (1968), with the main features of ideal wheat plant being determinate and synchronous growth habit, a short and strong stem, large leaf area and erect leaf, high harvest index and presence of awns. In the past few decades, gradual and continuous changes in climatic conditions have created a shift in crop plant characteristics (Fang et al., 2004). In a study conducted on wheat in China, overall duration of the growing period from germination to maturity and vegetative growth period from sowing to heading was found to be significantly decreased as a result of warming. In the case of the reproductive phase, the length of the growth period from heading to maturity was increased. These changes in the duration of each growth phase are due to the thermal requirements for the completion of different phenological phases (Tao et al., 2012).

The main purpose of modernizing the wheat ideotype was to ensure avoidance or tolerance of abiotic stresses by changing plant phenology, improved photosynthesis and staygreen trait of leaves during drought conditions (Semenov et al., 2014). In determinate cereals, vegetative and reproductive growth phases occur in sequence and hardly overlap on any single culm. While in case of indeterminate plants, vegetative and reproductive growth continue after fruiting has begun (Azam et al., 2002). The transition from vegetative growth to reproductive growth in plants is regulated by different external and internal factors. External signals regulating the flowering are day length (photoperiod) and temperature (vernalization), while internal factors are floral gene activities and developmental stage of a plant. Vernalization causes a developmental switch, turning off the leaf primordia production and starting the formation of floral primordia (Murai et al., 2003). Temperature also affects phase transitioning by initiating flowering, especially when there are low temperature conditions. A plant’s light harvesting potential, the synchrony of flowering and seed setting, tillering or branching and reproduction success is directly affected by the architecture of the shoot system (Kuraparthy et al., 2006). The number and geometry of plant organs are dependent on genetic inheritance and climatic conditions at the time of development. Studies have shown the improvement in carbon assimilation by altering plant architectural characteristics, which allows more light penetration throughout the plant, having important implications for yield (Sarlikioti et al., 2011)**.** Size, structure and other architectural properties cause the main differences in resource capture between short duration cereals and other plants. All structural attributes ultimately depend on the rates and duration of processes that control extension and strengthening of tissue (Azam et al., 2002). The different combinations of rate, duration and inherent branching pattern give rise to an extremely wide range of morphological crop types. The longevity and arrangement of the meristematic tissue determine the pattern of branching, the opportunity of assimilates storage and reproductive growth. Research on sole crops frequently deals with the efficient spatial capture and use of solar radiation; for example, by manipulating plant population or canopy architecture (Keating et al., 1993).

Vegetative meristems are partially or totally suppressed when flowering starts; any tendency to flower early also tends to reduce branching and indeterminate characteristics. In the case of indeterminate tillering, sterile tillers that contribute nothing to grain yield use resources such as light and water for their vegetative growth (Azam et al., 2002). Continuous water supply during the grain filling stage helps to maintain filling duration and grain size in heat stressed crops. Nevertheless, a reduction in single grain weight has still been seen (McDonald et al., 1983; Altenbach et al., 2003).

This study was conducted to determine the relationship between the growth habit of wheat plants and grain yield. Grain quality was further tested in relation to different growth types of plant and grain processing parameters. Moreover, vegetation indices were calculated using UAV multispectral data to monitor growth trends throughout the crop season.

3.2 Materials and methods

3.2.1 Plant material

The recombinant inbred lines (RILs) population developed from a cross between the heat tolerant wheat cultivar 'Halberd' and a moderate heat susceptible wheat cultivar 'Len' was planted in a Corpus Christi, TX field. The RILs population consists of 180 $F_{2:6}$ recombinant inbred lines (RILs). Two replications for each recombinant inbred line and parent lines Halberd and Len were planted in a 5x10 ft. plot in an alpha-lattice design.

3.2.2 Field data collection

The field data of the RILS population was collected, which included days to heading (from emergence to 50% heading), flowering time and duration, maturity time, plant height, tiller length range and grain yield. For plant growth habit, data for tiller height within the plant and phase of tillers formation relevant to vegetative and reproductive stages was recorded. The tillers length distribution for each RIL within a specific area under plantation was compared (Fig 3.1 A, B).

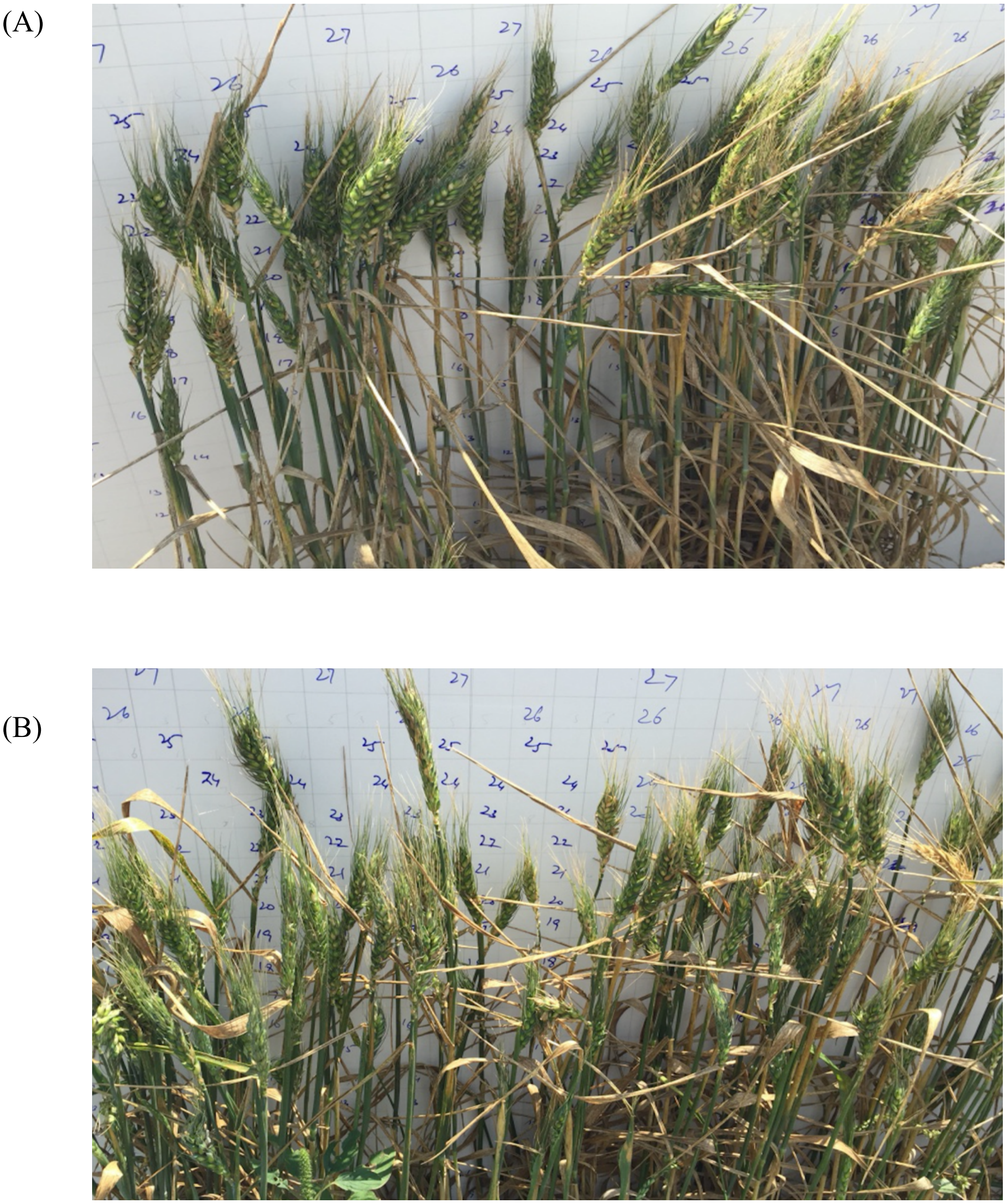

Figure 3.1 Comparison of plants for flowering and tillering pattern.

(A) Tillers and spikes are uniform in size in determinate plants. (B) Indeterminate wheat plant with asynchronized flowering and tillering pattern. Tillers and spikes are not uniform in size.

Plants with uniform flowering and tiller distribution with respect to height and phase of tillers formation were characterized as determinate while those with non-uniform flowering and tillers distribution were characterized as indeterminate. Grain yield of each RIL was harvested at $1ft^2$ area from the middle of the plot to have a true representative sample from the plot.

3.2.3 Single kernel hardness test

To compare grain quality with growth type, wheat milling quality assessment was performed to analyze processing quality. The single kernel characterization system (SKCS 4100) was used for wheat texture measurements.

Table 3.1 Characterization of wheat for hardness values determined by NIR.

Category	**NIR**
Extra Hard	Higher than 84
Very Hard	73-84
Hard	61-72
Medium hard	49-60
Medium Soft	37-48
Soft	25-36
Very Soft	13-24
Extra Soft	Lower than 12

This equipment works on the principle of recording the force required for crushing the kernel and reporting the results as the kernel hardness index (HI). The crushing force is directly affected by moisture content (%), kernel weight (mg) and diameter (mm). Grain hardness is mainly determined by near infrared spectroscopy (NIR). On the basis of NIR values, the standard scale used for characterizing the wheat grains into different categories is given in table 3.1.

3.2.4 Spectral reflectance data and vegetation indices calculation

A series of multispectral UAV images was taken from germination to maturity stage using a Tetracam ADC Snap imaging system equipped with RGB and NIR sensors. At each growth phase two field surveys were conducted for collecting imagery. The spatial resolution of the images was 2.5 cm with spectral 400 nm to 950 nm. Images were processed in ENVI software to examine trends in vegetation indices (NDVI, GNDVI and NIR/Green) for determinate and indeterminate group.

3.2.5 Statistical analysis

Data for plant growth type and agronomic traits was analyzed using JMP Pro to determine significance between determinate and indeterminate group for grain yield, quality parameters and vegetation indices in a replicated trial. A T-test was conducted to determine significance of grain yield difference between two groups. After finding significance of variations, a correlation test (Pearson) was conducted in R-Studio 1.0.136 to explore the relative associations between growth habit, grain yield single kernel parameters. The relative correlation coefficients were also calculated for vegetation indices and to test the association between VIs and grain yield.

3.3 Results

3.3.1 Characterization for flowering and tillering pattern

Distribution for determinate and indeterminate growth type in RILs on the basis of tiller length range with relative vegetative and reproductive phases is shown (Table 3.2). In determinate lines, flowering time was synchronized and tillers were uniform, while there was more variation in flowering time and tillers distribution among indeterminate lines. Determinate plants were early maturing as compared to the indeterminate line (Table 3.2).

Table 3.2 Distribution of RILs for determinate and indeterminate growth habit on the basis of flowering time, (when 50% of the heads were at anthesis; days after planting), tiller distribution within plant, distance between longer tillers and shorter tillers (cm).

	DETERMINATE	INDETERMINATE
NO. OF RILS	87	93
FLOWERING TIME	65-70 days	70-75 days
TILLERS LENGTH RANGE	12-15 cm	25-35 cm

3.3.2 Evaluation of RILs for grain yield

The grain yield was comparatively high in the case of determinate lines as compared to indeterminate lines. Figure 3.2 (Boxplot) illustrates the distribution response of the data points classified as determinate (Mean value=518.881 kg/acre, Std. Error= 18.821) and the data points classified as indeterminate (Mean value=427.623 kg/acre Std. Error=14.505), along with an outlier boxplot for context of data variation. This information suggests that mean intensity for grain yield between groups was significantly different. T-test confirmed the significant difference for yield between these two groups at P>0.05 (Table 3.3).

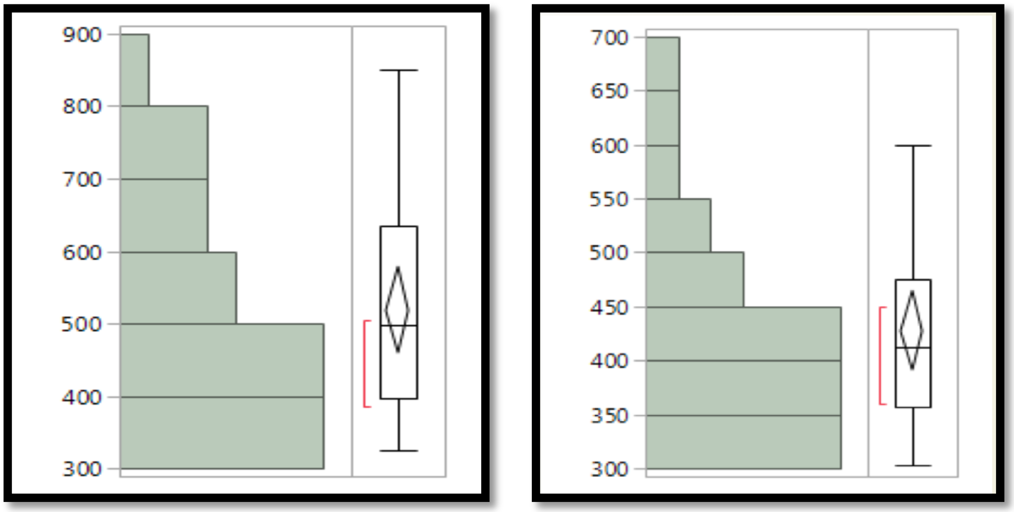

Figure 3.2 Distribution of high yielding determinate RILS (Group Mean = 518.881 kg/acre, Std. Error= 18.821) and high yielding indeterminate RILS (Group Mean = 427.623 kg/acre Std. Error=14.505) along with an outlier boxplot for context of yield data variation.

Table 3.3 T-test of significance for grain yield between determinate and indeterminate groups.

Difference	-91.26	t Ratio	-2.67978
Std Err Dif	34.05	DF	39.83977
Upper CL Dif	-22.42	Prob > |t|	0.0107*
Lower CL Dif	-160.09	Prob > t	0.9947
Confidence	0.95	Prob < t	0.0053*

3.3.2 Single kernel characterization measurements

The obtained SKCS data for Single kernel hardness, kernel diameter, kernel weight and kernel moisture content in determinate and indeterminate group is presented in table 3.4.

Table 3.4 Single kernel characterization system (SKCS4100) measurements.

Genotypes/Parameters		**Determinate**	**Indeterminate**
Hardness	Range	53.43 - 92.52	61.75 - 93.78
	Average	74.8	77.6
	S.D	8.71	6.72
Diameter (mm)	Range	2.13-2.75	2.06 - 2.66
	Average	2.39	2.3
	S.D	0.07	0.1

Table 3.4 Continued.

Genotypes/Parameters		**Determinate**	**Indeterminate**
Weight (mg)	Range	16.89-27.33	16.05-27.75
	Average	21.52	21.28
	S.D	2.2	1.98
Moisture (%)	Range	10.63-13.63	10.77-13.06
	Average	12.69	12.17
	S.D	2.49	0.54

3.3.4 Pearson's correlation coefficients

The correlation coefficients for grain yield related traits and their significant association is given in table 3.5.

Table 3.5 Pearson's correlation coefficients for grain yield related traits.

Only values assigned by symbol* are significant while rest of the coefficient values are non-significant.

Determinate

	WEIGHT	**DIAMETER**	**MOISTURE**	**YIELD**
WEIGHT				
DIAMETER	-0.10			
MOISTURE	-0.16	-0.01		
YIELD	0.50**	0.03	-0.10	

Table 3.5 Continued.

Indeterminate

	WEIGHT	DIAMETER	MOISTURE	YIELD
WEIGHT				
DIAMETER	0.17			
MOISTURE	0.15	0.09		
GRAIN YIELD	0.23*	0.63	0.61	

3.3.5 Temporal change in spectral reflectance

The calculated NDVI was slightly higher in determinate vs. indeterminate lines throughout the crop season, but it was not significant statistically. A similar trend was seen in ratio index NIR/Green, where values are consistent for both groups except for the heading stage, where it is significantly higher in the case of determinate plants. At four stages (flag leaf emergence, booting, heading and flowering), a significant difference was seen in GNDVI values between determinate and indeterminate group ($P < 0.05$). The GNDVI and NIR/Green index was almost similar at vegetative phases, but there is a slight increment in case of determinate lines at the early reproductive phase to the late reproductive phase (Figure 3.3).

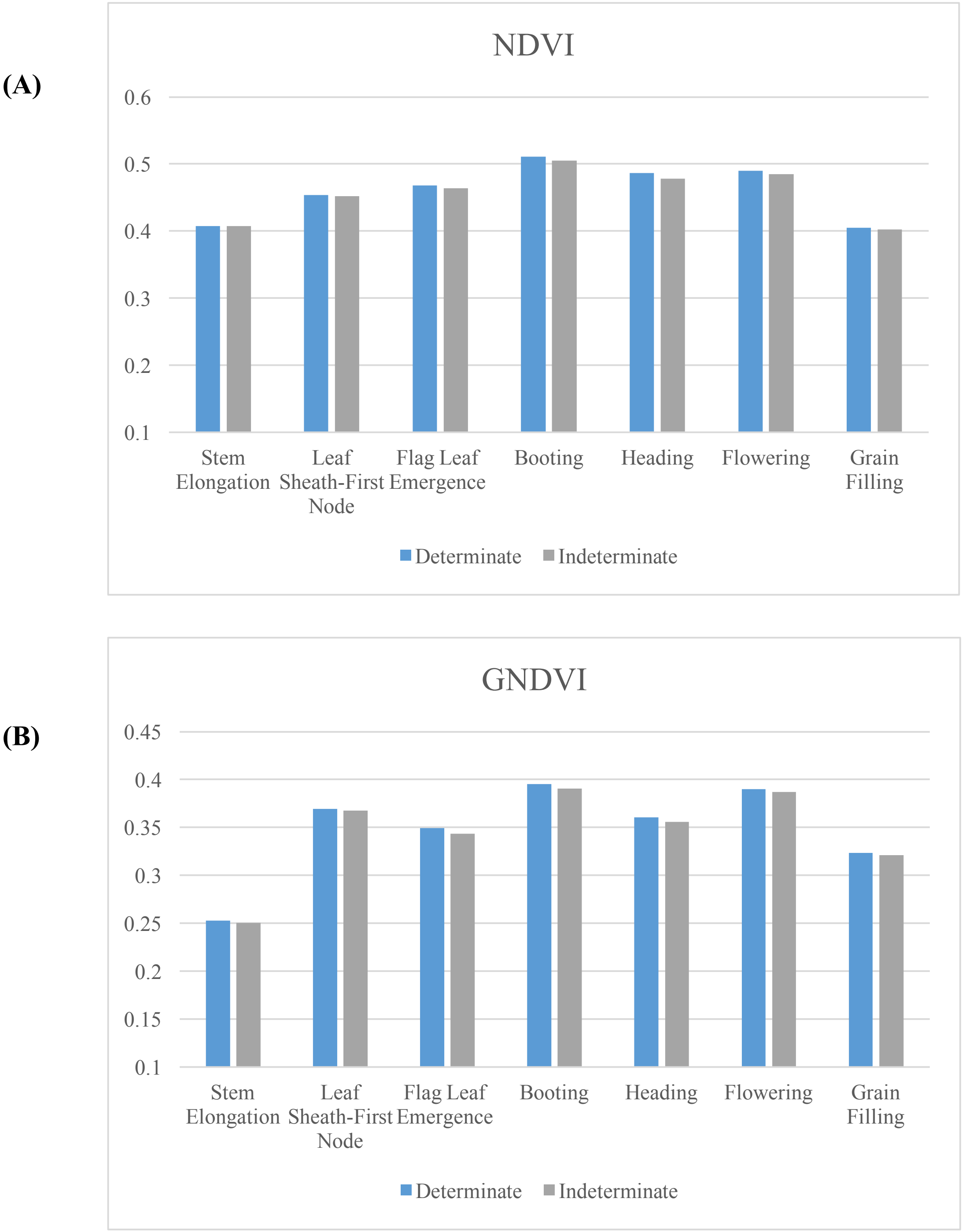

Figure 3.3 Vegetation indices trends for determinate and indeterminate RILS.

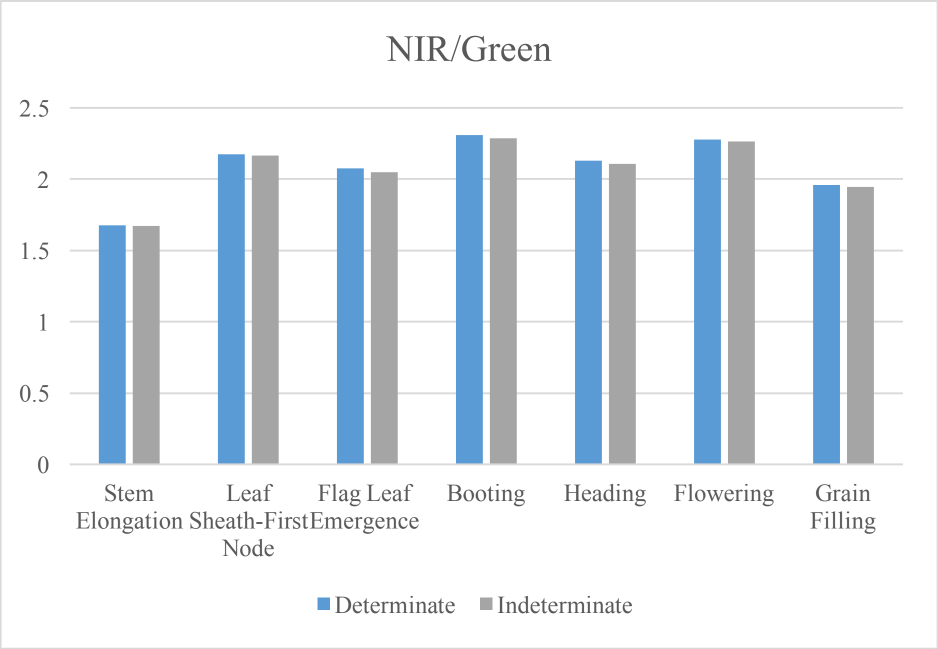

Figure 3.3 continued.

3.4 Discussion

Minimum vegetative growth and an earlier synchronized flowering, as in the case of determinate growth habit, results in a prolonged grain filling period during which sustained photosynthesis leads to production of sufficient assimilates to maximize size and weight and maintain quality of grains (Azam et al., 2002**).** Although increased temperature and photosynthesis rate enhances the assimilate supply and has a positive effect on grain protein content, it does not compensate completely for the short period of starch deposition. As a result, grains are small with low wheat processing quality (Nuttal et al., 2015, Li et al., 1995). In the current study, the determinate lines were early maturing with uniform flowering and tillering pattern. While in the case of indeterminate plants, the non-uniform tillering and flowering pattern resulted in variation in flowering time. The observed grain yield in determinate lines was significantly higher ($P \leq 0.05$). with a group mean of 518.881 kg/acre as compared to the indeterminate group with group mean of 427.623 kg/acre. This yield is extremely low as compared to typical yield. The reason behind this was late sowing (1^{st} week of February) followed by a series of rain falls followed by prolonged extreme temperatures (Av. Maximum 34 C°) during reproductive stages which severely affected grain yield. Our objective of this study was also to validate spectral reflectance data using multispectral UAV with plant growth pattern, therefore, if we see the VIs values, the highest value for NDVI is not more than 0.51 during crop season which is also an indication of influence of climatic effects and affected vegetation. The single kernel characterization parameters showed high grain weight and diameter in the case of determinate plants. A positive association was seen between Kernel weight and final grain yield ($P \leq 0.05$). The single kernel hardness test did not show a significant difference between the two groups. In the case of indeterminate tillering, sterile tillers that contribute nothing to grain yield use resources such as light and water for their vegetative growth (Azam et al., 2002). The NDVI index was slightly higher in the case of the determinate group throughout the period, but statistically there was no significant difference between the two groups. GNDVI and NIR/Green indices values were also marginally higher in case of determinate group as compared to indeterminate group.

CHAPTER IV

SUMMARY

In precision agriculture, UAVs may be used to monitor crop growth and health status which is less time taking as compared to using conventional phenotyping techniques. Using UAVs for monitoring crop status enables us to acquire images with high temporal resolution and high spatial resolution.

Climate change is a potential threat to ensure food security in the context of increasing food demand. Under stress conditions, biochemical signals within the plant for early branching prevent further increase in branches that results in uniform tillering pattern and flowering. Excessive tillering and vegetative growth is an undesirable trait in terms of harvesting and final grain yield and quality stability. Non-uniform flowering and tillering patterns affect maturation timing which in turn results in variability in grain size. In determinate plants, the defined pattern of vegetative growth and early completion of flowering results in a prolonged grain filling period. During prolonged grain filling stage the continuous supply of assimilated carbon maximizes the grain mass and size. The elevated temperature during the grain filling period, increases the assimilate supply but it does not compensate for the shorter grain filling period. A longer grain filling period can be ensured by delaying leaf senescence that is a key feature of the staygreen phenotype. A staygreen plant has an ability to maintain chlorophyll for a longer period of time during post anthesis stages. The chlorophyll retention that is associated with photosynthesis competence and ultimately provides carbon assimilates for a developing sink. The better strategy for selecting staygreen phenotype will help us to select plants that contribute in yield improvement. A challenge associated with measuring the staygreen trait is the lack of control of crop phenology when both the early and late maturing plants are being evaluated. Therefore, characterizing population into early and late maturing plants and use of the high throughput remote sensing technology for crop phenotyping for growth pattern may provide us better results to reduce bias between crop phenology and staygreen trait. By validating spectral reflectance vegetation indices including NDVI, GNDVI and ratio index NIR/green with staygreen plant phenology, we conclude that UAV multispectral data is useful in identifying the temporal changes in plant related to growth pattern.

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